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Research Article

DETERMINATION OF MEDIAN LETHAL DOSE OF TRIAZOPHOS WITH DMSO IN WISTAR RATS

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ABSTRACT

Objective: The present study was designed to evaluate the LD_{50} of triazophos (TZ), an organophosphorus pesticide in Wistar rats.

Materials and Methods: A single oral dose of TZ was dissolved in Dimethyl sulfoxide (DMSO) and administered orally at a concentration of 35, 55, 90 and 100 mg/kg respectively to experimental animals. The animals were observed for 2 hrs and then at 4th, 6th and 24th hr for any toxic signs and symptoms. After 24 hrs, the numbers of deceased rats in each group were counted, and percentage of mortality was calculated.

Results: LD_{50} of the TZ in DMSO was found to be 72.44 mg/kg. Single dose of TZ at 35 mg/kg did not reveal any toxic signs or behavioral alterations, hence it is considered as No Observed Adverse Effect Level (NOAEL) dose.

Conclusion: Knowledge of LD_{50} is very important for forensic toxicologists/toxicologist to correlate or to identify a substance or any poison and to measure the acute toxicity for drugs, food poisonings and accidental domestic poisonings cases. The knowledge gained from dose-response studies in animals is used to set standards for human exposure. Thus, the present study demonstrated for the LD_{50} of TZ using DMSO as a vehicle in Wistar rats. Very limited history is present in the case of pesticide poisoning and spectrum of poisoning in these cases is quite variable.

Keywords: Pesticides, Organophosphorus, Triazophos, Lethal dose 50, No observed adverse effect level.

INTRODUCTION

Pesticide substance or a mixture of substance used against pests in all development forms have become omnipresent contaminants of the environment and are also found in tissues of human and animal all over the world [1,2]. Pesticides are active substances that are directly released to the environment during and after the use phase of their life cycle. During the application, the mechanism for off-target movement is primarily direct aerial drift and volatilization. After deposition within the target site, numerous biological, physical and chemical processes determine the fate of the chemical [3]. There is a great range in the toxicity of pesticides to humans. The relative hazard of a pesticide is dependent upon the toxicity of the pesticide, the dose received, and the length of time exposed. A hazard can be defined as a source of danger. The hazard in using a pesticide is related to the likelihood of exposure to harmful amounts of the pesticide. Exposure can be influenced by the amount of pesticide used, concentration of the pesticide and how the pesticide and application equipment are handled. Pesticides have become an area of intense research due to its diverse properties and related effects. The demand for pesticide products and the concentration that they make towards agriculture efficiency are clear, but the volume of production indicates that the potential for misapplication and accidental exposure is great [4].

The toxicity of any chemical can be measured in several ways, but generally human toxicity is estimated based on test results on rats and other animal models. Any chemical or substance that is poisonous to rat is not necessarily equally poisonous to humans or other animals. Some pesticides are fatal after one large dose (acute toxicity); others can be dangerous after small, repeated doses (chronic toxicity). One simple measure of toxicity use bioassays to measure death rates in order to quantify the effect of the toxin. This measure is commonly known as a lethal dose 50 (LD_{50}). The LD_{50} is defined as the LD at which 50% of the population died in a given period under controlled and standardized laboratory conditions. LD_{50} is usually an initial screening step in the assessment and evaluation of the toxic characteristic of a substance. LD_{50} (median LD) is the statistically derived single dose of a substance which produces death in 50% of the population of test animals to which it is administered by any of the methods such as oral, dermal inhalation or intravenous. Determination of this test examines the relationship between dose and the most extreme response-Death. The more potent or toxic the chemical, lower the LD_{50} and the smaller the dose needed to cause death. Therefore, a pesticide with an oral LD_{50} of 500 mg/kg would be much less toxic than a pesticide with an LD_{50} of 5 mg/kg. Normally LD_{50} is expressed as mg/kg bw i.e., milligrams of substance per kilogram of animal body weight. Milligram (mg) and kilogram (kg) are metric units of weight. Milligrams per kilogram are the same as parts per million (ppm). It provides information on health hazards likely to arise from short-term exposure. This data serve as a basis for labeling and classification and also helpful in establishing a dosage regimen in sub-chronic and chronic studies.

We can calculate LD_{50} by various accepted methods for instance Miller and Tainter [5], Bliss [6], Litchfield and Wilcoxon [7], Finney [8], Weil [9] and Thompson [10].

Triazophos (TZ)(O, O-diethyl O-1-phenyl-1 H-1, 2,4-triazol-3-yl phosphorothioate) is a broad spectrum non-systemic organophosphorus pesticide (OP) used liberally throughout the world for plant protection [11-13]. The primary mechanism of action of TZ is neurotoxic and leads to accumulation of the neurotransmitter acetylcholine in synaptic, cholinergic and neuromuscular effects [14,15]. The oral LD_{50} of TZ in male Wistar rat according to some unpublished reports is 59 mg/kg bw in sesame oil [16] and 68 mg/kg bw in starch mucilage [17], according to the first draft of TZ toxicity [18]. Except World Health Organization report (WHO), no satisfactory data related to LD_{50} of TZ is available. Here an attempt was made to determine the oral LD_{50} of TZ pesticide in Wistar rats using DMSO as vehicle.

MATERIALS AND METHODS

Test chemicals

TZ Tech. grade (purity 62% EC) was obtained from Hindustan Insecticides Limited (A Government of India Enterprises).

Animals and experimental design

Twenty-five healthy adult male Wistar rats (weighing about 200±20 g) were obtained from Central Animal Facility (All India Institute of Medical Sciences, New Delhi, India). All the animals were allowed to acclimatize to the experimental conditions for a period of 5 days. All the rats were housed in polyacrylic cages, not more than three animals per cage and maintained under standard laboratory conditions (natural light/dark cycle, room temperature 22±3°C). Animals were given standard dry rat pellet diet and tap water was provided ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Dose preparation and administration

Rats were fasted for 18 hrs prior to dosing. The compound was administered once orally to the rats, using 22-gauze oral feeding needle. The volume of the dose depends on the size and weight of the animals. In rodents it should not exceed 1 ml/100 g body weight [1,19,20]. In this study, TZ was dissolved in 0.5 ml of DMSO.

Estimation of the dose range and percentage of mortalities

An approximate LD_{50} can be determined by the so-called "up and down" or the "staircase method" using two animals and increasing the doses of TZ [2]. Five doses were chosen which were given orally to five groups of rats, five rats in each group (Table 3), for the determination of LD_{50} of the TZ from 0% mortality to 100% mortality [21]. The animals were observed for 2 hrs and then at 4th, 6th and 24th hr for any toxic signs and symptoms. After 24 hrs, the numbers of deceased rats in each group were counted, and percentage of mortality was calculated using the graphical method of Miller and Tainter [5].

RESULTS

Signs recorded during experiment

Initially, the dose of TZ does not produce any significant effect on central nervous system at 35 mg/kg. However, at the doses 55, 90 and 100 mg/kg, animal showed signs of central nervous system stimulation for 16-24 hrs. The animals exhibited chewing, licking, salivation, arching and rolling, lacrimation, occasional pawing or burrowing, associated with movement of legs. The animals showed labored breathing, gasping and finally death within 7 days.

Conversion of percentage mortalities to probits and calculation of LD_{50}

The percentage of animals that died at each dose was then transformed to probit (Table 1) using Finney's method (Table 2). The percentage dead for 0 and 100 were corrected before the determination of probits. For 0% dead: 100 (0.25/n) and for 100% dead: 100 (n-0.25/n), where n=5 rats [19].

In the present case of TZ, Log LD_{50} is 1.86 and LD_{50} is 72.44 mg/kg. The SE of the LD_{50} was calculated using the following formula [19].

$$\text{Approximately SE of } LD_{50} = \frac{(\text{Log } LD_{84} - \text{Log } LD_{16})}{\sqrt{2N}} \quad \text{(i)}$$

The Probits of 84 and 16 from Table 2 are 5.99 and 4.01 (approximately 6 and 4), respectively. The log-LD values for the probits 6 and 4 are obtained from the line on the graph in Fig. 1, which, in the present case are 2.08 and 1.64 and their antilog are 120.23 and 41.67. Using these values in formula (i), The SE of LD_{50} is 17.64.

Therefore, LD_{50} of TZ with DMSO, when given orally was observed 72.44±17.64, with 95% confidence interval of 60.9-96.18.

Table 1: Graphical method of Miller and Tainter for TZ

Groups	Dose (mg/kg)	Log dose	Percentage of dead	Corrected percentage	Probits
1	0	0	0	0	0
2	35	1.54	0	5	3.36
3	55	1.74	40	40	4.75
4	90	1.95	60	60	5.25
5	100	2.00	80	80	5.84

The probit values thus obtained were plotted against log-dose and then the dose corresponding to probit five, i.e., 50% was found out (Fig. 1).
TZ: Triazophos

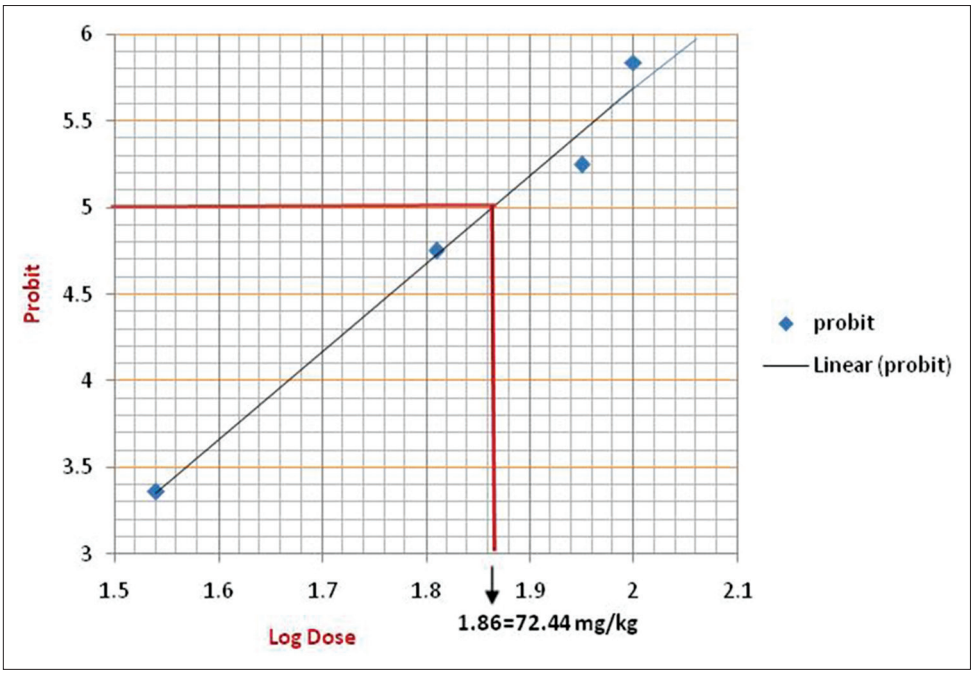

Fig. 1: Plot of log-doses versus probits from Table 2 for calculation of oral lethal dose 50 of triazophos

Table 2: Transformation of percentage mortalities to probit

Percentage	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Table 3: Dose of TZ given orally to rats

Groups	Number of animals	Dose of TZ in mg
1 (control)	5	-
2	5	35
3	5	55
4	5	90
5	5	100

TZ: Triazophos

DISCUSSION

Mankind has a history of using crop protection products, in the production of food supply and protection of the environment. Now days, pesticide plays a very important role in crop protection by providing dependable, persistent and relatively complete control against harmful pests with less labor and low cost. Pesticides are a very important group of environmental pollutants used in intensive agriculture for the protection against diseases and pests.

Pesticides may be classified by target organism, chemical structure and physical state, etc. Chemical structures differ within categories as well as between categories. Thus, toxicity to target and non-targeted species can vary widely within each group. Rampant use of pesticides in agricultural products continues to risk the life of the common man. Thus, it is very important to know the LD_{50} of the pesticide before using it in the fields [1].

Organophophorus compounds (Ops) are the most widely used pesticides worldwide and their metabolites are widespread across different populations [22-26]. Short-term effects of exposure to these chemicals have been studied mostly in the nervous system, which is their primary target, but there is a growing concern about their possible toxic effects in non-target tissues and chronic effects that have not been studied in such detail. The majority of people are continually exposed to low OP concentrations, and long-term epidemiologic studies reveal linkage to higher risk of cancer development [27,28]. The WHO estimates that every year 3 million people experience acute poisoning by Ops [29].

The primary mechanism of OPs toxicity is well studied-they function as inhibitors of the enzyme acetylcholinesterase (AChE). Like organochlorine derivatives, most of the organophosphate insecticides implicate the nervous system of the target organisms. They are capable of inhibiting the activity of the enzyme acetylcholine esterase which catalyses the removal of acetylcholine from the synaptic cleft after an impulse has passed through the junction. The inactivation of this enzyme causes acetyl choline to accumulate in the synaptic cleft which as a consequence remains in a charged state blocking further transmission of nerve impulses through the cleft [30].

Rampant use of pesticide in agriculture products continue to risk of environmental pollution as well as the life of the common man [1]. Thus, it is very important to know the LD_{50} of the pesticide before using it in the fields, excessive dose of pesticide may cause acute toxicity. Therefore, the present study was conducted to find out the LD_{50} of the OP pesticide TZ. It did not show any gross visible changes and toxic signs at 35 mg/kg so; this dose is considered to be the no observed adverse effect level (NOAEL). However, animals administered with TZ at the doses of 55, 90 and 100 mg/kg showed a sequence of the signs of toxicity viz. acute cholinergic symptoms, chewing, licking, salivation, writhing, arching and rolling, lacrimation, occasional pawing or burrowing, hyperactivity to sound/touch, abnormal gait pattern, incoordinaton, imbalance, difficulty breathing, anxiety, ataxia, depression of respiration and circulation, and convulsions etc. after the dosing and these symptoms were persisted for 6 hrs. The pattern of the signs after the administration of TZ is strongly suggestive of AChE inhibition.

The geographic mean of these two doses (a) the lowest dose which had killed one animal, and (b) the highest dose which did not kill any animal, determines LD_{50} of a particular substance. The graphical representation of probit versus log dose showed a typical straight line which was in agreement with the principle of probit analysis (Fig. 1). According to Miller and Tainter probit analysis method [1], at 24 hr the acute oral LD_{50} value of TZ in DMSO was calculated as 72.44 mg/kg (with 95% confidence interval) in the present study, which is 1.23 folds higher than the LD_{50} of TZ in Sesame oil and 1.06 folds higher than the LD_{50} of TZ in Starch mucilage. The reason behind this may be using of DMSO as a solvent and purity of TZ (62% tech grade). DMSO is used as a vehicle because, it is particularly a good solvent and is non-hydroxylic and so it is less likely to react with the pesticides. Manna *et al.* [31] also used DMSO as a vehicle in his study and reported that DMSO reduces toxicity of the pesticide which means an increase in the value of LD_{50}. Actual decrease in LD_{50} or increases in mortality is used to assess the scale of the increase in toxicity following exposure and vice versa.

However, published experimental work on TZ toxicity in rat is limited and there is no experimental work reported on the TZ toxicity with DMSO as a vehicle. However, it is concluded that, the overall result of the present study clearly demonstrates that oral LD_{50} of the TZ using vehicle DMSO is as 72.44 mg/kg bw (with 95% confidence interval) and NOAEL of the combination of the above mentioned pesticide is 35 mg/kg bw in Wistar rats.

CONCLUSION

Knowledge of LD_{50} is very important for forensic toxicologists/ toxicologist to correlate or to identify a substance or any poison and to measure the acute toxicity for drugs, food poisonings and accidental domestic poisonings cases. The knowledge gained from dose-response studies in animals is used to set standards for human exposure. Since, very less work is reported in case of TZ pesticide. Thus, the present study demonstrated for the first time the LD_{50} of TZ using DMSO as a vehicle in Wistar rats. Very limited history is present in the case of pesticide poisoning and spectrum of poisoning in these cases are quite variable. No specific guidelines are practice for toxicological analysis in such cases. Hence understanding of median LD and toxicity is very important for the better evaluation of the toxic characteristic of TZ and also to measure its short-term poisoning potential.

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REFERENCES

1. Raj J, Chandra M, Dogra TD, Pahuja M, Raina A. Determination of median lethal dose of combination of endosulfan and cypermethrin in wistar rat. Toxicol Int 2013;20(1):1-5.
2. Anwar WA. Biomarkers of human exposure to pesticides. Environ Health Perspect 1997;105 Suppl 4:801-6.
3. Available from: http://www.bestchoicemall.com/howtomakecompostguide/pesticide%20cycle.gif. [Last accessed on 2013 Sep 18].
4. Environmental Information System-National Institute of Occupational Health 2007;2:1. (Available from http://sgh.org.sa/Portals/Articles/Occupational and Environmental Health-Recognizing and Preventing Disease and Injury.pdf. [Last accessed on 2013 Sep 18].
5. Miller LC, Tainter ML. Estimation of LD_{50} and its error by means of log - Probit graph paper. Proc Soc Exp Biol Med 1944;57:261-4.
6. Bliss CI. The method of probits – A correction. Science 1934;79(2053):409-10.
7. Litchfield JT Jr, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 1949;96(2):99-113.
8. Finney DJ. Probit Analysis. 3rd ed. Cambridge, UK: Cambridge University Press; 1971.
9. Weil CS. Tables for convenient calculation of median effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics 1951;8(3):249-63.
10. Thompson WR. Use of moving averages and interpolation to estimate median-effective dose; fundamental formulas, estimation of error, and relation to other methods. Bacteriol Rev 1947;11(2):115-45.
11. Mohineesh C, Raina A, Raj J, Dogra TD. Development of new solvent systems for the analysis of triazophos (O, O-diethyl O-1-phenyl-1H-1, 2, 4-triazol-3-yl phosphorothioate) extracted from blood. Proc Indian Natl Sci Acad 2012;78(1):34.
12. Jain S, Mythily S, Ahmed RS, Arora VK, Banerjee BD. Induction of oxidative stress and histopathological changes by sub-chronic doses of triazophos. Indian J Biochem Biophys 2010;47(6):388-92.
13. Mingjing Q, Zhaojun H, Xinjun X, Lina Y. Triazophos resistance mechanisms in the rice stem borer (Chilo suppressalis Walker). Pestic Biochem Physiol 2003;77(3):99-105.
14. Kamanyire R, Karalliedde L. Organophosphate toxicity and occupational exposure. Occup Med (Lond) 2004;54(2):69-75.
15. Singh M, Rishi S. Plasma acetylcholinesterase as a biomarker of triazophos neurotoxicity in young and adult rats. Environ Toxicol Pharmacol 2005;19(3):471-6.
16. Hollander H, Weigand W. Testing for acute intraperitoneal toxicity in the male SPF wistar rats. Unpublished Report No. 77.0364 from Hoechst AG, Pharma Development, Submitted to WHO by Aventis CropScience, Frankfurt am Main, Germany; 1977. Available from (http://sgh.org.sa/Portals/Articles/Occupational and Environmental Health-Recognizing and Preventing Disease and Injury.pdf. [Last accessed on 2013 Sep 18].
17. Hollander H, Weigand W. Acute oral toxicity to the male SPF-wistar rat. Unpublished Report No. 77.0360 from Hoechst AG, Pharma Research Toxicology, Submitted to WHO by Aventis Crop Science, Frankfurt Main, Germany; 1977. (Available from http://sgh.org.sa/Portals/Articles/Occupational and Environmental Health-Recognizing and Preventing Disease and Injury.pdf. [Last accessed on 2013 Sep 18].
18. Available from: http://www.inchem.org/documents/jmpr/jmpmono/2002pr14.htm. [Last accessed on 2013 Sep 18].
19. Ghosh MN. Fundamentals of Experimental Pharmacology. 2nd ed. Calcutta, India: Scientific Book Agency; 1984. p. 153-8.
20. Turner R. Quantal responses calculation of the ED_{50}. Screening Methods in Pharmacology. New York: Academic Press; 1965. p. 61-3.
21. Randhawa MA. Calculation of LD50 values from the method of Miller and Tainter, 1944. J Ayub Med Coll Abbottabad 2009;21(3):184-5.
22. Aprea C, Strambi M, Novelli MT, Lunghini L, Bozzi N. Biologic monitoring of exposure to organophosphorus pesticides in 195 Italian children. Environ Health Perspect 2000;108(6):521-5.
23. Barr DB, Bravo R, Weerasekera G, Caltabiano LM, Whitehead RD Jr, Olsson AO, *et al.* Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population. Environ Health Perspect 2004;112(2):186-200.
24. Curl CL, Fenske RA, Elgethun K. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. Environ Health Perspect 2003;111(3):377-82.
25. Kattavenkatesh R, Kumar N. Study the assessment of poisoning cases in a rural tertiary care teaching hospital by a clinical pharmacist. Asian J Pharm Clin Res 2012;5(2):138-41.
26. Heikal TM, Mossa AT, Rasoul MA, Marei GK. The ameliorating effect of green tea extract against cyromazine and chlorpyrifos induced liver toxicity in male rats. Asian J Pharm Clin Res 2013;6(1):48-55.
27. Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, *et al.* Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res 1990;50(20):6585-91.
28. Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF, *et al.* Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). Cancer Causes Control 2001;12(6):509-17.
29. World Health Organization. Public Health Impact of Pesticides Used in Agriculture. Geneva: WHO; 1990. Available from: http://whqlibdoc.who.int/publications/1990/9241561394.pdf. [Last accessed on 2014 Aug 04].
30. Riviere JE. Dermal absorption/toxicity of organophosphates and carbamates. In: Gupta RC, editor. Toxicology of Organophosphate and Carbamate Compound. New York: Elsevier Academic Press; 2006.
31. Manna S, Bhattacharya D, Basak DK, Mandal TK. Single oral dose toxicity of α-cypermethrin in rats. Indian J Pharmacol 2004;36(1):25-8.